Horizontal Gene Transfer and Genome Evolution in the Phylum *Actinobacteria*

Cooper J. Park, Joshua T. Smith, and Cheryl P. Andam

Abstract *Actinobacteria* constitute one of the largest phyla in the bacterial domain, with many species known for their large genome sizes and high G + C content. Members of this phylum exhibit a wide range of morphological, physiological, and metabolic properties and are ubiquitously distributed in diverse ecological niches. The phylum includes pathogens, symbionts of plants and invertebrates, gastrointestinal commensals, and free-living bacteria. Many species are well known for their ability to produce a plethora of natural products relevant to agriculture, biotechnology, and medicine, including majority of naturally derived antibiotics widely used in clinics. In this chapter, we discuss recent advances that highlight the role of horizontal gene transfer (HGT) in the evolutionary history, genomic structure, and ecological diversity of *Actinobacteria*, with emphasis on HGT between species and between phyla. We highlight genome studies that describe how HGT has contributed to the success of promiscuous antibiotic producers as well as the pathogens that they target. We conclude with how methods in population genomics can be used to elucidate the impacts of selective pressures and environmental changes on rates and patterns of HGT in various *Actinobacteria* species and populations.

Keywords Horizontal gene transfer · Genome · Evolution · *Actinobacteria* · Biosynthetic gene clusters

1 Introduction

Actinobacteria have long been the major source of novel naturally derived antibiotics and analogs widely used today in clinical practice. The drug discovery revolution began in 1944, when the biochemist and soil microbiologist Selman Waksman described for the first time the isolation of streptomycin, the first effective treatment

C. J. Park · J. T. Smith · C. P. Andam (✉)
Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH, USA
e-mail: Cheryl.Andam@unh.edu

© Springer Nature Switzerland AG 2019

T. G. Villa, M. Viñas (eds.), *Horizontal Gene Transfer*,
https://doi.org/10.1007/978-3-030-21862-1_5

for tuberculosis, from the soil-dwelling *Actinobacteria Streptomyces griseus* (Schatz et al. 1944). Tuberculosis is a highly infectious disease caused by another actinobacterium *Mycobacterium tuberculosis*. Long considered the scourge of humanity, tuberculosis has, from ancient times, caused some of the deadliest epidemics ever seen in human history. Today, the disease remains a major public health threat, especially in Asia, where two million deaths are reported annually (Zumla et al. 2015). Waksman would go on to describe a variety of other valuable antibiotics produced by diverse *Streptomyces* species (Kutzner and Waksman 1959; Woodruff 2014). Since that time, enormous research effort has been directed toward this genus, and today more than half of all clinically useful bioactive compounds are derived from *Streptomyces*. While *Streptomyces* is the most well-known genus in the field of natural drug discovery, a diverse number of *Actinobacteria* have also been reported to be equally if not more prolific in producing compounds, enzymes, and other natural products relevant to medicine, agriculture, biotechnology, and energy production. Among these are members of the genera *Amycolatopsis*, *Micromonospora*, *Nocardia*, *Pseudonocardia*, *Saccharomonospora*, and *Salinispora* (Barka et al. 2015). Beyond antibiotics, multitude of compounds (known as secondary metabolites) derived from actinobacterial biosynthetic gene clusters (BGCs) have also been reported to be effective antifungals, antivirals, antitumors, anthelmintics, antiprotozoals, and immunosuppressants (Běhal 2000; Manivasagan et al. 2014).

The importance of *Actinobacteria* goes beyond the pharmaceutically relevant bioactive compounds they produce. In addition to the deadly *M. tuberculosis* and *Mycobacterium leprae* (causal agent of leprosy), many other species in this phylum cause devastating diseases in humans and animals (e.g., *Corynebacterium*, *Nocardia*, *Propionibacterium*, *Rhodococcus*, *Tropheryma*) (Barka et al. 2015). *Actinobacteria* are ubiquitously distributed in both aquatic and terrestrial ecosystems and play a critical role in ecosystem functions and biotic interactions. For example, *Actinobacteria* comprise the defensive mutualists of animals (e.g., *Pseudonocardia*, *Streptomyces*), gastrointestinal commensals (e.g., *Bifidobacterium*), soil inhabitants (e.g., *Micromonospora*, *Streptomyces*), marine dwellers (e.g., *Salinispora*), plant mutualists (e.g., nitrogen-fixing *Frankia*), and plant pathogens (e.g., scab-forming *Streptomyces*) (Ventura et al. 2007; Barka et al. 2015). Because many *Actinobacteria* species are able to degrade insoluble polymers such as cellulose and chitin, they also contribute significantly to the global carbon cycle through the breakdown of plant biomass (Anderson et al. 2012; Book et al. 2014).

The ecological, industrial, and biomedical importance of *Actinobacteria* cannot be overstated. However, the taxonomy of *Actinobacteria* has long been considered problematic, with species membership constantly being modified, reclassified, and improperly named (Embley and Stackebrandt 1994; Stackebrandt and Schumann 2006). This is especially true for *Streptomyces* in the early days of its discovery, when species were classified and named based on the antibiotics produced, rapidly escalating the number of species to over 3000 (Trejo 1970). The taxonomy of other genera of *Actinobacteria* has been historically difficult to resolve, with numerous incongruencies between phenotypic and genotypic groupings and named species (Embley and Stackebrandt 1994; Stackebrandt and Schumann 2006). With the

availability of genome sequences in the past two decades, it has become possible to reconstruct a more reliable and robust picture of the evolutionary history of *Actinobacteria* (Ventura et al. 2007; Nouioui et al. 2018), although majority of actinobacterial genomes sequenced so far belong to organisms relevant to human and veterinary medicine, biotechnology, and industry.

Genome-based studies of *Actinobacteria* reveal reticulate evolutionary processes due to horizontal gene transfer (HGT) and are likely to blur the boundaries between species (Ventura et al. 2007), producing metaspecies and fuzzy species with ambiguous species boundaries (Hanage et al. 2005). HGT can result in genetically mosaic genomes with genes having disparate origins which may be different from the history of the organism itself and therefore are difficult to classify (Gogarten et al. 2002; Bapteste and Boucher 2009; Corander et al. 2012). HGT is known to have shaped the evolutionary history and diversity of *Actinobacteria* (Fig. 1). Even in species long thought to be extremely clonal (e.g., *M. tuberculosis*), recent genome-wide studies indicate that they are not immune to HGT (Panda et al. 2018). Hence, we find that the ecological success of *Actinobacteria* in a wide variety of environments, from marine sediments and soil to the gut of eukaryotic hosts, has been shaped by their ability to gain and donate DNA. In this chapter, we highlight recent genome-based studies that explore their evolutionary history of the phylum *Actinobacteria* and the important role that HGT has played in generating their tremendous genetic, ecological, and biosynthetic diversity.

2 HGT in Free-Living, Antibiotic-Producing *Streptomyces* and *Salinispora*

Salinispora The marine-dwelling *Salinispora*, composed of three species *Salinispora arenicola*, *Salinispora tropica*, and *Salinispora pacifica*, is a rich source of structurally diverse natural products (Jensen et al. 2015). Although *Salinispora* spp. share >99% 16S rRNA gene sequence similarity (Jensen and Mafnas 2006) making species delineation is challenging, species-specific patterns of secondary metabolite production and gene distributions may likely contribute to speciation in the genus (Jensen et al. 2007; Freel et al. 2011; Ziemert et al. 2014). For example, rifamycins and saliniketals were produced exclusively by *S. arenicola* species and are the main secondary metabolites differentiating *S. arenicola* and *S. pacifica* (Bose et al. 2014).

Genome sequencing of *Salinispora* strains reveals that a large part of the genome is dedicated to BGCs and natural product assembly (Penn et al. 2009). For example, approximately 10% of the genome of *S. tropica* comprise a variety of polyketide synthases, nonribosomal peptide synthetases, and several hybrid clusters, with the majority being novel BGCs (Udwary et al. 2007). *S*pecies harbor an average of 14–18 polyketide and nonribosomal peptide BGCs per strain, but only <10% of the total predicted BGCs are conserved or thought to be shared by the common ancestor

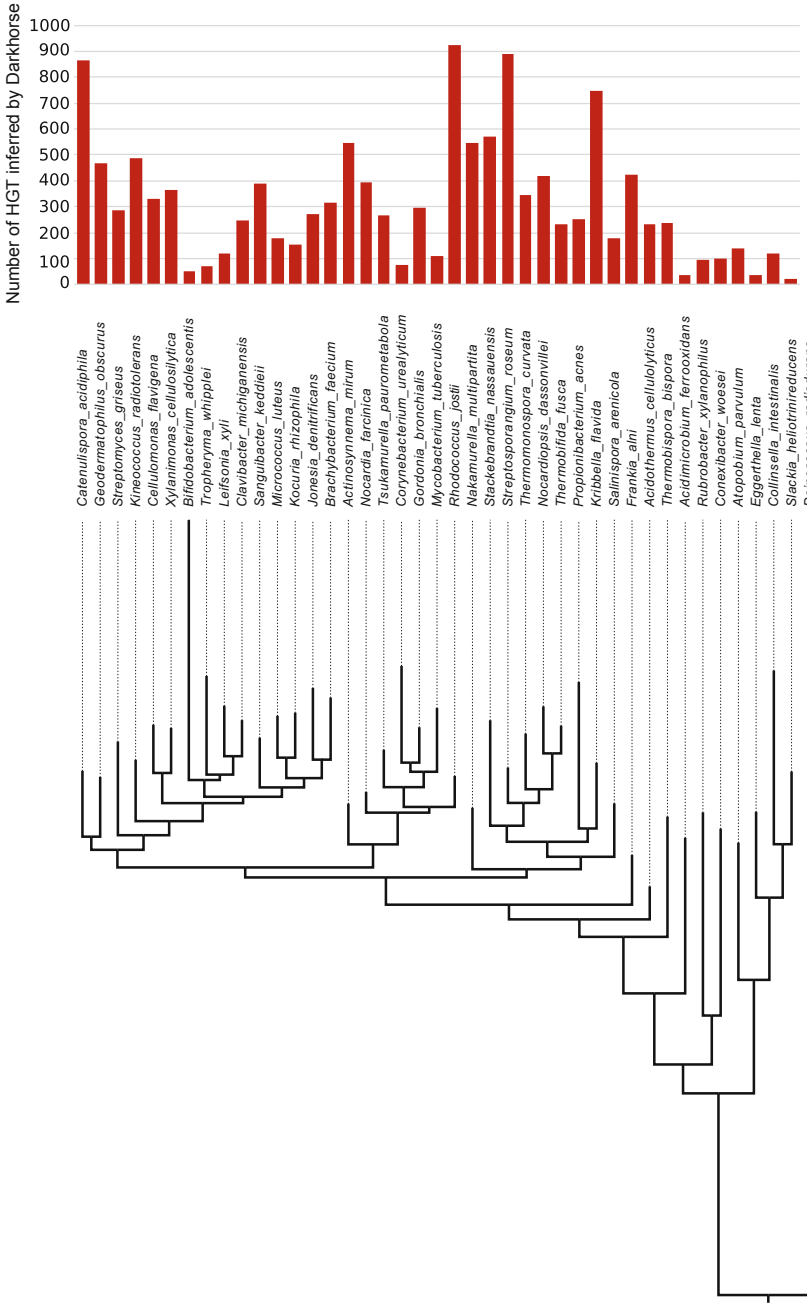

Fig. 1 Number of genes in *Actinobacteria* inferred to have experienced HGT. Left: Maximum likelihood phylogenetic tree of the 16S rRNA locus of representative *Actinobacteria* species. The phylogeny was built using PhyML (Guindon et al. 2010), with 100 bootstrap replicates, GTR nucleotide substitution model (Tavaré 1986) + I + G. Sequences were aligned using MUSCLE (Edgar 2004). *Deinococcus radiodurans* and *Thermus thermophilus* were used as outgroup taxa to root the tree. Right: HGT events were inferred using Darkhorse v.2 (Podell et al. 2008), which calculates a lineage probability index (LPI) based on BLAST scores and the phylogenetic distance of the match sequence from the query organism

of the genus (Ziemert et al. 2014). More than half of the BGCs are rare and are present in only one or two strains, suggesting that they were acquired relatively recently in the evolutionary history of *Salinispora* (Ziemert et al. 2014). Acquired BGCs are often incorporated into genomic islands that are frequently exchanged within and between *Salinispora* species and have evolved by gene gain, loss, and duplication followed by divergence (Udwary et al. 2007; Ziemert et al. 2014).

HGT of BGCs in *Salinispora* has greatly contributed to its tremendous chemical diversity and highlights a population-level strategy to secondary metabolite production. First, the same genomic island can encode one to five distinct BGCs depending on the strain and that the identity of a BGC within a specific island can vary dramatically between strains (Ziemert et al. 2014). While their exchange may be random, this diversity of BGC in the same chromosomal position may reflect a plug-and-play model of evolution that allows the strain to test the relative fitness effects of specialized metabolites that it produces (Letzel et al. 2017). Second, transposase-mediated HGT of BGCs can result in molecular diversification of a single class of small molecules as was observed in salinosporamide A and K in *S. tropica* and *S. pacifica*, respectively (Eustáquio et al. 2011; Freel et al. 2011). Third, functional redundancy resulting from HGT can also occur, as was observed in the BGC encoding for the peptidic siderophores called salinichelins, which were acquired in three independent HGT events throughout the evolutionary history of *Salinispora* (Bruns et al. 2018). The acquisition and retention of the salinichelin siderophores co-occurred with the loss of the more ancient desferrioxamine pathway. Lastly, the high number of strain-specific BGCs may be a population-level strategy that prevents the permanent loss of a BGC from the population by maintaining them in one or few strains. When the necessity arises (e.g., environmental change, competition with other organisms), these rare BGCs may be disseminated among other members of the population via recombination. Overall, the different ways by which *Salinispora* strains mix and match their BGCs suggest that the genus is able to maximize its population-level diversity and distribution of secondary metabolites while restricting the number of BGCs maintained within any individual genome (Ziemert et al. 2014). This may be a case of BGCs representing a shared genomic resource, built on the premise of the Black Queen Hypothesis wherein cells produce only a fraction of the common goods and rely on other cells to produce the others (Fullmer et al. 2015). BGC distribution, mobility, and structural rearrangement within and between *Salinispora* species can consequently have an immediate influence on fitness, on niche utilization, and consequently on bacterial diversification (Penn et al. 2009). This has been observed in *Salinispora* wherein BGCs exhibit high levels of species specificity and can be used to differentiate closely related but ecologically distinct populations (Penn et al. 2009).

Streptomyces *Streptomyces* constitutes a highly diverse bacterial group that is widely distributed in nature. In contrast to most bacteria, *Streptomyces* species are characterized by complex secondary metabolism and a fungal-like morphological differentiation that involves the formation of branching, filamentous vegetative growth, and aerial hyphae bearing long chains of reproductive spores (Flärdh and

Buttner 2009); hence they were originally misclassified as fungi. *Streptomyces* genomes are one of the largest in the bacterial domain, ranging from 8 to 11 Mb and are characterized by a large portion of accessory genes (Harrison and Studholme 2014). Genome analyses indicate that this remarkable genome structure has been shaped by widespread HGT not just with other *Streptomyces* lineages but also with other microbial phyla (Ventura et al. 2007; Zhou et al. 2012).

HGT of partial and entire BGCs is known to play a major role in generating the high number and variety of BGCs in *Streptomyces* genomes (Egan et al. 2001). In the marine-dwelling MAR4 lineage of *Streptomyces*, a total of 13 distinct kinds of hybrid isoprenoids (HIs) BGCs are recognized, each of which is predicted to encode the production of a distinct HI compounds (Gallagher and Jensen 2015). This chemical diversity has been postulated to have arisen as a result of the frequent HGT of ABBA prenyltransferases (PTases) in other *Streptomyces* lineages, which are enzymes responsible for the attachment of isoprene moieties to a variety of acceptor molecules, a key step in HI biosynthesis (Gallagher and Jensen 2015). Widespread duplication and HGT of PTases among unrelated BGCs may also explain the relatively high number of ABBA PTase homologs in all of the MAR4 genomes, which average five copies per strain, compared to other *Streptomyces* species which carry one copy per strain (Gallagher and Jensen 2015). Recipients of *Streptomyces* DNA are not limited to other *Actinobacteria* genera. For example, the antibiotic bicyclomycin, the only naturally derived antibiotic that selectively inhibits the transcription termination factor Rho, is synthesized by *Streptomyces cinnamoneus* (Miyoshi et al. 1972; Skordalakes et al. 2005). HGT of intact BGC encoding bicyclomycin is facilitated by mobile genetic elements, and transfer can span across phylogenetically distant taxa within *Actinobacteria* as well as numerous members of *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* (Vior et al. 2018). Other *Streptomyces*-derived BGCs have also been reported to have had a history of HGT, such as those that encode the antibiotics streptomycin (Tolba et al. 2002), foxicin (Greule et al. 2017), antimycin (Joynt and Seipke 2018), and granaticin (Deng et al. 2011), as well as toxins such as coronafacoyl phytotoxins (Bown et al. 2017) and tunicamycin (Sechler et al. 2017).

Strain-level variation in genomic and BGC content has only recently been recognized and highlights the importance of how recent HGT events can impact recent evolutionary divergence among closely related strains. Multiple *Streptomyces* species with identical 16S rRNA gene sequences and considered the same species can exhibit distinct secondary metabolomes (Antony-Babu et al. 2017). In *Streptomyces albus*, a promiscuous producer of numerous clinically relevant compounds, a total of 48 BGCs were identified in a mere 7 strains (Seipke 2015). Of these, 18 specify the core BGC of the species, 14 are auxiliary BGCs, and 16 are strain-specific (Seipke 2015). Similar results were also reported in two sister-taxa of *Streptomyces* inhabiting distinct geographic ranges demarcated by latitude. Variation in BGC content and consequently divergence between them are reported to have been driven primarily by both recent HGT and gene loss events (Choudoir et al. 2018).

The rate in which genes are horizontally acquired and retained in *Streptomyces* lineages may not necessarily reflect widespread and rampant HGT and instead may have been influenced by the length of time that has occurred since the origins of the genus. This was demonstrated by McDonald and Currie who used a molecular clock and showed that HGT is rare in *Streptomyces*, with an estimated average of 10 genes per million years that have been horizontally acquired and retained (McDonald and Currie 2017). The authors argue that the high number of HGT events in large bacterial genomic datasets may be due to the long periods of time that separate sampled strains (McDonald and Currie 2017). Even with few housekeeping genes, often used in multilocus sequence analyses (MLSA), similar results have been reported. The fixation of ancestrally recombined genes in *Streptomyces* can result in an indefinitely large number of contemporary lineages that have inherited "horizontally acquired" genes through vertical descent (Andam et al. 2016). However, observed low rates of HGT may only be limited to core genes, but BGCs and other accessory genes are likely to exhibit recent recombination more frequently.

The unique chromosomal structure in *Streptomyces* genomes is likely to explain the large pan-genomes and frequent HGT in the genus. *Streptomyces* genomes consist of a large linear chromosome, with a conserved central region flanked by highly variable arms characterized by large DNA rearrangements (Bentley et al. 2002; Ikeda et al. 2003). Double-strand break repair and nonhomologous end joining (NHEJ) in the chromosome can result in major chromosome structure alteration and promote HGT (Hoff et al. 2018). Moreover, widespread interspecies HGT can be mediated by the pool of highly diverse actinophages (Sharaf et al. 2017). Some of these actinophages have remarkably mosaic genome structure, suggesting frequent exchange with other phages. For example, the actinophages of *Streptomyces flavovirens* Sf1 and Sf3 share 100% identity in conserved regions interspersed between highly variable regions with high variability (with sequence similarity ranging from 23.9 to 87.5%) (Sharaf et al. 2017). Lastly, *Streptomyces* also utilize a unique conjugative DNA transfer mechanism directed by the enzyme TraB, a plasmid-encoded DNA-translocase that transfers a double-stranded DNA-molecule to the recipient (Thoma and Muth 2015) [in contrast, other conjugation machineries transfer only single-stranded DNA (Grohmann et al. 2018)]. A multi-protein complex then spreads the transferred plasmid to neighboring mycelial compartments of the recipient, resulting in the rapid dissemination of the donor DNA (Thoma et al. 2015).

3 HGT in the Pathogens *Mycobacterium*, *Rhodococcus*, and *Corynebacterium*

Mycobacterium The genus *Mycobacterium* comprises more than 180 species of which about 15 deadly pathogens are commonly encountered in humans and animals (Gupta et al. 2018). The extent of HGT and its impacts on the evolution of

Mycobacterium remain controversial (Godfroid et al. 2018), particularly so in the human-restricted *M. tuberculosis* which have long been considered clonal, immune to HGT, and evolving only by random genetic drift and selection (Hershberg et al. 2008; Gagneux 2018). However, recent genome-wide studies of different *Mycobacterium* species indicate that *Mycobacterium* genomes had undergone many episodes of intra- and interspecies transfer of genes from taxonomically diverse sources (Boritsch et al. 2016; Panda et al. 2018).

Horizontally acquired genes have been reported to have played a major role in the pathogenesis of *Mycobacterium* species. In a genome-wide analysis of HGT in *Mycobacterium* using the program T-REX which infers probable HGTs based on the statistical reconciliation of gene and species trees (Boc et al. 2012), genes acquired from outside of the genus have been reported to be integral in the emergence of its antibiotic resistance, virulence, and other metabolic pathways (Panda et al. 2018). The major donor clades include other genera in the phylum *Actinobacteria* (*Rhodococcus*, *Gordonia*, *Segniliparus*, *Streptomyces*, and *Tsukamurella*) with some genes also donated by *Proteobacteria*. *Mycobacterium* phages Sbash and Whirlwind (*Siphoviridae*) also appear to be frequent partners (Panda et al. 2018). However, there is increasing evidence that HGT has been more frequent early in the evolution of the genus, and perhaps this might explain the long-held view that HGT is rare in *Mycobacterium*. An ancient HGT event has been reported to have contributed to the ability of *M. tuberculosis* to persist for long periods of time in lung hypoxic lesions, which is a major factor in the high burden of latent tuberculosis (Levillain et al. 2017). The ancient HGT event involves the gene cluster for the synthesis of the molybdenum cofactor, which allows an organism to respire nitrate and to persist in a dormant state under hypoxia, a stress condition encountered in lung tuberculosis lesions (Williams et al. 2014). This HGT occurred after the divergence from environmental non-tuberculous mycobacteria but before the clonal expansion of the *M. tuberculosis* clonal complex, with the most likely donor being closely related to plasmids of the betaproteobacterium *Burkholderia vietnamensis* (Levillain et al. 2017). Ancient genomic islands also persist and continue to be mobilized by conjugative plasmids and phages among mycobacteria species (Reva et al. 2015).

Interspecies HGT within the genus appears to be common in many other species of *Mycobacterium*. For example, *Mycobacterium abscessus* is an emerging, multidrug-resistant, non-tuberculous mycobacterium responsible for a wide spectrum of infections (skin and soft tissue diseases, central nervous system infections, bacteremia, and ocular infections). It is composed of three subspecies: *abscessus*, *massiliense*, and *bolletii.* Studies show that while HGT through homologous recombination is prevalent within the species, the rate of DNA acquisition is asymmetrical among the three subspecies (Sapriel et al. 2016). Genome sequences of hybrid strains indicate that horizontally acquired large genomic blocks with sizes up to 93 Kb are distributed throughout their genomes and are likely acquired through punctuated massive HGT events (Sapriel et al. 2016). What is more worrisome is that these genomically hybrid strains are significantly associated with macrolide resistance and with cystic fibrosis patients with lung infections or chronic

colonization (Sapriel et al. 2016). In *Mycobacterium avium* subsp. *hominissuis*, genomic islands carrying genes associated with virulence, antibiotic resistance, and fitness are highly variable and are shared among different *Mycobacterium* species (Sanchini et al. 2016). There is also evidence of HGT in the genomes of other subspecies of *M. avium* (*avium*, *silvaticum*, and *paratuberculosis*) (Uchiya et al. 2017). Even core housekeeping genes have also been reported to have been transferred between *Mycobacterium* species, as in the case of a recombinant *rpoB* in some *Mycobacterium yongonense* strains gained from *Mycobacterium parascrofulaceum* (Kim et al. 2017a) and *rpoB* in some *M. massiliense* strains acquired from *Mycobacterium abscessus* (Kim et al. 2017b).

Genome-wide mosaicism in *Mycobacterium* created through HGT within and between species is likely mediated by distributive conjugal transfer (DCT), a newly described form of chromosomal conjugation that is not driven by conjugative plasmids and the origin of transfer (*oriT*) sequence as in other conjugation processes (Gray et al. 2013). Through chromosomal transfer, DCT results in a mosaic transconjugant structure, similar to the products of meiosis in eukaryotes (Gray et al. 2013). Genome sequencing of transconjugants of *Mycobacterium smegmatis* reveal that up to a quarter of the genome, distributed over 30 segments with sizes ranging from 50 bp to 225 Kb, has been derived horizontally (Derbyshire and Gray 2014). DCT has also been observed in another species, *Mycobacterium canettii*, a close relative of *M. tuberculosis* that has been isolated on rare occasions from cases of tuberculosis in East Africa (Mortimer and Pepperell 2014). While more data is needed to explain the mechanism of transfer, it appears to involve the co-transfer of multiple chromosome segments into the recipient after fragmentation in the donor cell before transfer, followed by recombination in the recipient chromosome to generate a mosaic pattern (Derbyshire and Gray 2014).

Rhodococcus Rhodococci are aerobic and nonmotile soil organisms that can be found in a variety of environments such as soil, water, and insect guts (Bell et al. 1998). While most species are free-living and saprophytic, two are known to be pathogenic. *Rhodococcus equi* is a pulmonary pathogen of young horses and AIDS patients (von Bargen and Haas 2009; Topino et al. 2010), while *Rhodococcus fascians* causes leafy gall syndrome in more than 120 plant species representing both monocots and dicots, herbaceous and woody plants (Putnam and Miller 2007).

Conjugative plasmids are essential in the virulence and host adaptation of pathogenic *Rhodococcus* species. In *R. equi*, conjugative plasmids confer the ability to survive and replicate inside host macrophages (von Bargen and Haas 2009). Three types of plasmids have been identified: the circular equine-associated pVAPA and porcine-associated pVAPB and the linear bovine-associated pVAPN. The vap pathogenicity island (PAI) in the plasmids consists of multiple genes encoding virulence-associated proteins called vaps (Letek et al. 2008; MacArthur et al. 2017). pVAPN is a 120-kb invertron-like linear replicon unrelated to the two other plasmids and has been reported to have originated via HGT of the vap PAI to a linear replicon (Valero-Rello et al. 2015). Invertrons are mobile genetic elements composed of DNA with inverted terminal repeats at both ends, which can vary from 10 to

1000 bases (Sakaguchi 1990). Although highly diverse, the distribution of the three types of plasmids is strongly correlated with the animal host but can be exchanged and hence can bring about corresponding host shifts (Anastasi et al. 2016; MacArthur et al. 2017). It is also notable that the pathogenicity of *R. equi* evolved through the recruitment or co-option of core actinobacterial traits, following the horizontal acquisition of key host-adaptive genes (Letek et al. 2010). Gene co-option (also known as preadaptation or exaptation) involves rapid adaptive changes by taking traits that have evolved for one purpose and using them in a new context with new roles (True and Carroll 2002).

The linear plasmid pFiD188 is essential to the virulence of the plant pathogen *R. fascians* (Francis et al. 2012). Although majority of genes in the *R. fascians* chromosome appear to have not experienced frequent HGT, acquisition of only four pFiD188-encoded functions (secondary metabolism [*att*], gene transcription [*fasR*], cytokinin biosynthesis [*fasD*], and cytokinin activation [*fasF*]) is sufficient for *Rhodococcus* to infect plants (Creason et al. 2014). This is because the *fas* operon encodes the enzymatic machinery for the production of cytokinins, which cause hormonal imbalance in plants, resulting in abnormal growth (Pertry et al. 2009). Hence, plasmid-mediated HGT can rapidly convert nonpathogenic *R. fascians* strains into phytopathogenic variants.

Corynebacterium *Corynebacterium* species are members of the normal human microflora, often isolated from the skin, mucous membranes, and gastrointestinal tract (Bernard 2012; Sangal and Hoskisson 2016). However, they are now increasingly being recognized as causing opportunistic diseases particularly among individuals who are immunocompromised, have prosthetic devices, or have stayed in hospitals or nursing homes for long periods of time (Funke et al. 1997). HGT mediated by PAI and mobile elements is known to play an important role in the virulence and antibiotic resistance of pathogenic *Corynebacterium* species.

The most clinically significant species is *Corynebacterium diphtheriae*, the causal agent of diphtheria, a highly infectious disease of the throat and upper airways, through the production of a potent bacteriophage-encoded exotoxin diphtheria toxin (*tox*) (Holmes 2000). The *tox* gene and another major virulence factor (pilus), which play important roles in adhesion of *C. diphtheriae* to different host tissues (Mandlik et al. 2007), are found in genomic islands that are mobilizable between strains. Even more alarming is that the number of pilus gene clusters present in a strain and the variation introduced by gene gain or loss significantly correlate with variation in adhesive and invasive properties in *C. diphtheriae* strains (Sangal and Hoskisson 2016). Moreover, genes that encode resistance against common antibiotics can also be rapidly disseminated in the bacterial population. For example, in the antibiotic-resistant *C. diphtheriae* BH8, the genes that encode chloramphenicol, sulfonamides, and tetracyclines are carried in a gene region flanked by insertion sequences and can be horizontally acquired via transpositional integration (Trost et al. 2012).

HGT has also been reported in other pathogenic *Corynebacterium* species. In *Corynebacterium striatum* isolated from immunocompromised patients with underlying respiratory diseases, genome analysis reveals resistance genes mobilized by

IS3504 and ISCg9a-like insertion sequences (Nudel et al. 2018). Moreover, the presence of resistance genes flanked by unpaired insertion sequences suggests that a single recombination event could rapidly disseminate resistance to antibiotics aminoglycosides, macrolides, lincosamides, and tetracyclines in the bacterial population (Nudel et al. 2018). In another example, *Corynebacterium pseudotuberculosis* is a facultative intracellular pathogen and causes several infectious and contagious chronic diseases in goat, sheep, buffalo, and horses (Baraúna et al. 2017). The major toxin of *C. pseudotuberculosis* is phospholipase D encoded by the *pld* gene and is carried by a PAI PiCp1 (McKean et al. 2007; Ruiz et al. 2011). Fifteen other PiCp types, which contain several virulence factors such as genes that encode for fimbrial subunits, adhesion factors, iron uptake, and secreted toxins (Ruiz et al. 2011), are known to be present and are shared within and among the different *C. pseudotuberculosis* biovars (Baraúna et al. 2017; Viana et al. 2017).

4 *Actinobacteria* as HGT Donors in Microbial Communities

Actinobacteria are ubiquitous in different habitats and are major players in the functioning of many microbial communities. The abundance of diverse *Actinobacteria* and their ability to mobilize genetic material suggest that they can facilitate the rapid and widespread dissemination of antibiotic resistance genes across multiple taxa (D'Costa et al. 2006). This is because antibiotic resistance genes are often encoded on the same BGCs that encode the synthesis of antibiotics and other bioactive compounds. Antibiotic-producing bacteria carry resistance genes as a self-protecting mechanism toward the antibiotics they produce (Hopwood 2007) or to modulate their own signaling activity (Goh et al. 2002; Sengupta et al. 2013). Hence, it has long been postulated that *Actinobacteria* can transfer their BGC-associated resistance genes to other taxa (Benveniste and Davies 1973), but only after advances in metagenomic sequencing do we find significant and widespread evidence of this.

HGT between *Actinobacteria* and other microbial taxa in a community highlights the role of this phylum as a major reservoir of antibiotic resistance genes that can be widely disseminated to other phyla, including clinically relevant pathogens. Multidrug-resistant soil-dwelling bacteria that harbor resistance cassettes against five classes of antibiotics (beta-lactams, aminoglycosides, amphenicols, sulfonamides, and tetracyclines) exhibit perfect nucleotide identity to genes from diverse human pathogens, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Salmonella enterica* (Forsberg et al. 2012). Transfer of resistance genes is facilitated by integrases and transposases adjacent to the resistance genes (Forsberg et al. 2012). Other mechanisms of transfer may be prevalent in soil communities. For example, the transient nature of broad host range plasmids provides a direct, rapid link in transferring DNA between Gram-negative (*Proteobacteria*) and Gram-positive bacteria (*Actinobacteria* and *Firmicutes*) in soil communities (Klümper et al. 2015). Lastly, unidentified or undiscovered taxa that act as intermediates can

facilitate an indirect transfer of resistance genes between *Actinobacteria* and other phyla but remain invisible to current HGT detection methods. We also have to recognize that these resistance genes have had a long history of diversification that began long before the era of clinical antibiotics (D'Costa et al. 2011). It is likely that HGT events in the soil may have been more prevalent in the past than it is today. In soil bacteria inhabiting pristine and remote Antarctic surface soils, a total of 177 naturally occurring genes conferring resistance to antibiotics were reported to have originated from antibiotic-producing bacteria including *Actinobacteria*. These resistance genes were subsequently disseminated through ancient HGT events and have since been vertically inherited over many generations in the recipient lineages (Van Goethem et al. 2018).

While HGT is expected to occur more frequently between more closely related taxa (Andam and Gogarten 2011; Bolotin and Hershberg 2017), a possible mechanism that can mediate inter-phyla transfer of resistance genes has been recently proposed between *Actinobacteria* and *Proteobacteria* (Jiang et al. 2017). Called carry-back, this mechanism involves the conjugative transfer of a carrier sequence from *Proteobacteria* to *Actinobacteria*, followed by transposon-mediated recombination of the carrier sequence with the resistance gene in the *Actinobacteria*. This results in the formation of a sandwich structure of actinobacterial DNA flanked by proteobacterial DNA. Upon the death of the *Actinobacteria* cell, the sandwich-structured DNA is released and taken up by neighboring *Proteobacteria* that integrates the full resistance-transposon sequence into its own DNA. The carry-back mechanism has been demonstrated to have caused the transfer of two genes conferring resistance against chloramphenicol and lincomycin from *Streptomyces* to pathogenic *Proteobacteria* such as *Pseudomonas*, *Klebsiella*, and *Enterobacter* (Jiang et al. 2017). How prevalent this mechanism is across different *Actinobacteria* taxa, or between *Actinobacteria* and other phyla besides *Proteobacteria*, remains to be explored.

5 HGT and Genomic Heterogeneity Within Species

Microbial species are remarkably heterogeneous. They may be clonal, but microbial populations are often composed of multiple co-circulating lineages distinguished by phenotypic and genetic differences (Sánchez-Romero and Casadesús 2014; Davis and Isberg 2016; Takhaveev and Heinemann 2018). Such tremendous genomic heterogeneity leads to the pan-genome, defined as the totality of all unique gene families found in a group of genomes, often many times larger than a single genome (Medini et al. 2005). This variation in gene content can drive species divergence (Papke et al. 2007; Youngblut et al. 2015), metabolic diversity and versatility (Silby et al. 2011), and symbiotic relationships (De Maayer et al. 2014). Results from the few population-level studies of *Actinobacteria* reveal enormous inter-strain genetic and phenotypic variation (Doroghazi and Buckley 2014; Antony-Babu et al. 2017;

Garcia et al. 2018; Neuenschwander et al. 2018), but the evolutionary and ecological factors that drive this variation remain poorly understood.

Gene content variability among strains of the same species is largely attributed to both differential gene loss and HGT (Snel et al. 2002; McInerney et al. 2017; Hall et al. 2017; Bobay and Ochman 2018). The power of microbial population genomics, involving sequencing of hundreds and even thousands of closely related isolates of the same species, has revealed an astonishing level of genomic diversity both in terms of allelic differences and gene content variation (Whitaker and Banfield 2006; Vinatzer et al. 2014; Sheppard et al. 2018). Strain-level genomic heterogeneity can also provide valuable insights into how rates and patterns of HGT are impacted by selective pressures due to recent environmental changes such as climate change, host switching, and clinical interventions. While population genomics has been more commonly used to explore pathogen populations with implications for transmission studies, treatment regimens, and new drug target development (Wilson 2012), in *Actinobacteria*, such analyses have been widely used only in *M. tuberculosis* (Lee et al. 2015; Comas et al. 2015; Stucki et al. 2016; Otchere et al. 2018). However, population genomics is seldom employed in studies of other pathogenic species of *Actinobacteria* and environmental populations. *Actinobacteria* species offers an excellent model to explore within-species heterogeneity in a variety of environments, whether they be within a single eukaryotic host or between host species, in cryptic ecological niches, and across large geographical distances.

6 Conclusions and Future Outlook

We have only discerned a small fraction of the mechanisms, impacts, and barriers of HGT in *Actinobacteria* species. There is much yet to be discovered, and one promising approach to address these topics is population genomics. The ability of *Actinobacteria* to donate and gain DNA has tremendously influenced its survival in a variety of environments since its ancient origin. Recent comparative studies reveal that the genomic repertoires of actinobacterial species and populations are in constant flux as a result of widespread HGT, changing in response to the local environment, clinical interventions, and microbial neighbors. *Actinobacteria* use a variety of mechanisms to acquire DNA from their environment and from other cells, from mobile genetic elements to double-stranded DNA conjugation and phages. Acquired DNA can be mixed and matched in BGCs to generate diverse chemical inventory that a species can potentially produce, while resistance and virulence genes can be transferred to create deadly pathogens. In microbial communities, *Actinobacteria* are important HGT donors, disseminating genes with potential selective advantage in recipient lineages.

References

Anastasi E, MacArthur I, Scortti M et al (2016) Pangenome and phylogenomic analysis of the pathogenic actinobacterium Rhodococcus equi. Genome Biol Evol 8:3140–3148. https://doi.org/10.1093/gbe/evw222

Andam CP, Gogarten JP (2011) Biased gene transfer in microbial evolution. Nat Rev Microbiol 9:543–555. https://doi.org/10.1038/nrmicro2593

Andam CP, Choudoir MJ, Vinh Nguyen A et al (2016) Contributions of ancestral inter-species recombination to the genetic diversity of extant Streptomyces lineages. ISME J 10:1731–1741. https://doi.org/10.1038/ismej.2015.230

Anderson I, Abt B, Lykidis A et al (2012) Genomics of aerobic cellulose utilization systems in Actinobacteria. PLoS One 7:e39331. https://doi.org/10.1371/journal.pone.0039331

Antony-Babu S, Stien D, Eparvier V et al (2017) Multiple Streptomyces species with distinct secondary metabolomes have identical 16S rRNA gene sequences. Sci Rep 7:11089. https://doi.org/10.1038/s41598-017-11363-1

Bapteste E, Boucher Y (2009) Epistemological impacts of horizontal gene transfer on classification in microbiology. Methods Mol Biol 532:55–72. https://doi.org/10.1007/978-1-60327-853-9_4

Baraúna RA, Ramos RTJ, Veras AAO et al (2017) Assessing the genotypic differences between strains of Corynebacterium pseudotuberculosis biovar equi through comparative genomics. PLoS One 12:e0170676. https://doi.org/10.1371/journal.pone.0170676

Barka EA, Vatsa P, Sanchez L et al (2015) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1–43. https://doi.org/10.1128/MMBR.00019-15

Běhal V (2000) Bioactive products from Streptomyces. Adv Appl Microbiol 47:113–156

Bell KS, Philp JC, Aw DW, Christofi N (1998) The genus Rhodococcus. J Appl Microbiol 85:195–210

Bentley SD, Chater KF, Cerdeño-Tárraga A-M et al (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 417:141–147. https://doi.org/10.1038/417141a

Benveniste R, Davies J (1973) Aminoglycoside antibiotic-inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. Proc Natl Acad Sci U S A 70:2276–2280

Bernard K (2012) The genus corynebacterium and other medically relevant coryneform-like bacteria. J Clin Microbiol 50:3152–3158. https://doi.org/10.1128/JCM.00796-12

Bobay L-M, Ochman H (2018) Factors driving effective population size and pan-genome evolution in bacteria. BMC Evol Biol 18:153. https://doi.org/10.1186/s12862-018-1272-4

Boc A, Diallo AB, Makarenkov V (2012) T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. Nucleic Acids Res 40:W573–W579. https://doi.org/10.1093/nar/gks485

Bolotin E, Hershberg R (2017) Horizontally acquired genes are often shared between closely related bacterial species. Front Microbiol 8:1536. https://doi.org/10.3389/fmicb.2017.01536

Book AJ, Lewin GR, McDonald BR et al (2014) Cellulolytic Streptomyces strains associated with herbivorous insects share a phylogenetically linked capacity to degrade lignocellulose. Appl Environ Microbiol 80:4692–4701. https://doi.org/10.1128/AEM.01133-14

Boritsch EC, Khanna V, Pawlik A et al (2016) Key experimental evidence of chromosomal DNA transfer among selected tuberculosis-causing mycobacteria. Proc Natl Acad Sci U S A 113:9876–9881. https://doi.org/10.1073/pnas.1604921113

Bose U, Hewavitharana AK, Vidgen ME et al (2014) Discovering the recondite secondary metabolome spectrum of Salinispora species: a study of inter-species diversity. PLoS One 9:e91488. https://doi.org/10.1371/journal.pone.0091488

Bown L, Li Y, Berrué F et al (2017) Biosynthesis and evolution of coronafacoyl phytotoxin production in the common scab pathogen Streptomyces scabies. Appl Environ Microbiol 83(19):AEM.01169-17. https://doi.org/10.1128/AEM.01169-17

Bruns H, Crüsemann M, Letzel A-C et al (2018) Function-related replacement of bacterial siderophore pathways. ISME J 12:320–329. https://doi.org/10.1038/ismej.2017.137

Choudoir MJ, Pepe-Ranney C, Buckley DH (2018) Diversification of secondary metabolite biosynthetic gene clusters coincides with lineage divergence in Streptomyces. Antibiotics (Basel) 7:12. https://doi.org/10.3390/antibiotics7010012

Comas I, Hailu E, Kiros T et al (2015) Population genomics of Mycobacterium tuberculosis in Ethiopia contradicts the virgin soil hypothesis for human tuberculosis in sub-Saharan Africa. Curr Biol 25:3260–3266. https://doi.org/10.1016/j.cub.2015.10.061

Corander J, Connor TR, O'Dwyer CA et al (2012) Population structure in the Neisseria, and the biological significance of fuzzy species. J R Soc Interface 9:1208–1215. https://doi.org/10.1098/rsif.2011.0601

Creason AL, Vandeputte OM, Savory EA et al (2014) Analysis of genome sequences from plant pathogenic Rhodococcus reveals genetic novelties in virulence loci. PLoS One 9:e101996. https://doi.org/10.1371/journal.pone.0101996

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377. https://doi.org/10.1126/science.1120800

D'Costa VM, King CE, Kalan L et al (2011) Antibiotic resistance is ancient. Nature 477:457–461. https://doi.org/10.1038/nature10388

Davis KM, Isberg RR (2016) Defining heterogeneity within bacterial populations via single cell approaches. BioEssays 38:782–790. https://doi.org/10.1002/bies.201500121

De Maayer P, Chan WY, Rubagotti E et al (2014) Analysis of the Pantoea ananatis pan-genome reveals factors underlying its ability to colonize and interact with plant, insect and vertebrate hosts. BMC Genomics 15:404. https://doi.org/10.1186/1471-2164-15-404

Deng M-R, Guo J, Li X et al (2011) Granaticins and their biosynthetic gene cluster from Streptomyces vietnamensis: evidence of horizontal gene transfer. Antonie Van Leeuwenhoek 100:607–617. https://doi.org/10.1007/s10482-011-9615-9

Derbyshire KM, Gray TA (2014) Distributive conjugal transfer: new insights into horizontal gene transfer and genetic exchange in mycobacteria. Microbiol Spectr 2:MGM2-0022–MGM2-2013. https://doi.org/10.1128/microbiolspec.MGM2-0022-2013

Doroghazi JR, Buckley DH (2014) Intraspecies comparison of Streptomyces pratensis genomes reveals high levels of recombination and gene conservation between strains of disparate geographic origin. BMC Genomics 15:970. https://doi.org/10.1186/1471-2164-15-970

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

Egan S, Wiener P, Kallifidas D, Wellington EM (2001) Phylogeny of Streptomyces species and evidence for horizontal transfer of entire and partial antibiotic gene clusters. Antonie Van Leeuwenhoek 79:127–133

Embley TM, Stackebrandt E (1994) The molecular phylogeny and systematics of the actinomycetes. Annu Rev Microbiol 48:257–289. https://doi.org/10.1146/annurev.mi.48.100194.001353

Eustáquio AS, Nam S-J, Penn K et al (2011) The discovery of salinosporamide K from the marine bacterium "Salinispora pacifica" by genome mining gives insight into pathway evolution. Chembiochem 12:61–64. https://doi.org/10.1002/cbic.201000564

Flärdh K, Buttner MJ (2009) Streptomyces morphogenetics: dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol 7:36–49. https://doi.org/10.1038/nrmicro1968

Forsberg KJ, Reyes A, Wang B et al (2012) The shared antibiotic resistome of soil bacteria and human pathogens. Science 337:1107–1111. https://doi.org/10.1126/science.1220761

Francis I, De Keyser A, De Backer P et al (2012) pFiD188, the linear virulence plasmid of Rhodococcus fascians D188. Mol Plant-Microbe Interact 25:637–647. https://doi.org/10.1094/MPMI-08-11-0215

Freel KC, Nam S-J, Fenical W, Jensen PR (2011) Evolution of secondary metabolite genes in three closely related marine actinomycete species. Appl Environ Microbiol 77:7261–7270. https://doi.org/10.1128/AEM.05943-11

Fullmer MS, Soucy SM, Gogarten JP (2015) The pan-genome as a shared genomic resource: mutual cheating, cooperation and the black queen hypothesis. Front Microbiol 6:728. https://doi.org/10.3389/fmicb.2015.00728

Funke G, von Graevenitz A, Clarridge JE, Bernard KA (1997) Clinical microbiology of coryneform bacteria. Clin Microbiol Rev 10:125–159

Gagneux S (2018) Ecology and evolution of Mycobacterium tuberculosis. Nat Rev Microbiol 16:202–213. https://doi.org/10.1038/nrmicro.2018.8

Gallagher KA, Jensen PR (2015) Genomic insights into the evolution of hybrid isoprenoid biosynthetic gene clusters in the MAR4 marine streptomycete clade. BMC Genomics 16:960. https://doi.org/10.1186/s12864-015-2110-3

Garcia SL, Stevens SLR, Crary B et al (2018) Contrasting patterns of genome-level diversity across distinct co-occurring bacterial populations. ISME J 12:742–755. https://doi.org/10.1038/s41396-017-0001-0

Godfroid M, Dagan T, Kupczok A (2018) Recombination signal in Mycobacterium tuberculosis stems from reference-guided assemblies and alignment artefacts. Genome Biol Evol 10:1920–1926. https://doi.org/10.1093/gbe/evy143

Gogarten JP, Doolittle WF, Lawrence JG (2002) Prokaryotic evolution in light of gene transfer. Mol Biol Evol 19:2226–2238

Goh E-B, Yim G, Tsui W et al (2002) Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. Proc Natl Acad Sci U S A 99:17025–17030. https://doi.org/10.1073/pnas.252607699

Gray TA, Krywy JA, Harold J et al (2013) Distributive conjugal transfer in mycobacteria generates progeny with meiotic-like genome-wide mosaicism, allowing mapping of a mating identity locus. PLoS Biol 11:e1001602. https://doi.org/10.1371/journal.pbio.1001602

Greule A, Marolt M, Deubel D et al (2017) Wide distribution of Foxicin biosynthetic gene clusters in Streptomyces strains - an unusual secondary metabolite with various properties. Front Microbiol 8:221. https://doi.org/10.3389/fmicb.2017.00221

Grohmann E, Christie PJ, Waksman G, Backert S (2018) Type IV secretion in gram-negative and gram-positive bacteria. Mol Microbiol 107:455–471. https://doi.org/10.1111/mmi.13896

Guindon S, Dufayard J-F, Lefort V et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010

Gupta RS, Lo B, Son J (2018) Phylogenomics and comparative genomic studies robustly support division of the genus Mycobacterium into an emended genus Mycobacterium and four novel genera. Front Microbiol 9:67. https://doi.org/10.3389/fmicb.2018.00067

Hall JPJ, Brockhurst MA, Harrison E (2017) Sampling the mobile gene pool: innovation via horizontal gene transfer in bacteria. Philos Trans R Soc Lond Ser B Biol Sci 372:20160424. https://doi.org/10.1098/rstb.2016.0424

Hanage WP, Fraser C, Spratt BG (2005) Fuzzy species among recombinogenic bacteria. BMC Biol 3:6. https://doi.org/10.1186/1741-7007-3-6

Harrison J, Studholme DJ (2014) Recently published Streptomyces genome sequences. Microb Biotechnol 7:373–380. https://doi.org/10.1111/1751-7915.12143

Hershberg R, Lipatov M, Small PM et al (2008) High functional diversity in Mycobacterium tuberculosis driven by genetic drift and human demography. PLoS Biol 6:e311. https://doi.org/10.1371/journal.pbio.0060311

Hoff G, Bertrand C, Piotrowski E et al (2018) Genome plasticity is governed by double strand break DNA repair in Streptomyces. Sci Rep 8:5272. https://doi.org/10.1038/s41598-018-23622-w

Holmes RK (2000) Biology and molecular epidemiology of diphtheria toxin and the tox gene. J Infect Dis 181(Suppl 1):S156–S167. https://doi.org/10.1086/315554

Hopwood DA (2007) How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? Mol Microbiol 63:937–940. https://doi.org/10.1111/j.1365-2958.2006.05584.x

Ikeda H, Ishikawa J, Hanamoto A et al (2003) Complete genome sequence and comparative analysis of the industrial microorganism Streptomyces avermitilis. Nat Biotechnol 21:526–531. https://doi.org/10.1038/nbt820

Jensen PR, Mafnas C (2006) Biogeography of the marine actinomycete Salinispora. Environ Microbiol 8:1881–1888. https://doi.org/10.1111/j.1462-2920.2006.01093.x

Jensen PR, Williams PG, Oh D-C et al (2007) Species-specific secondary metabolite production in marine actinomycetes of the genus Salinispora. Appl Environ Microbiol 73:1146–1152. https://doi.org/10.1128/AEM.01891-06

Jensen PR, Moore BS, Fenical W (2015) The marine actinomycete genus Salinispora: a model organism for secondary metabolite discovery. Nat Prod Rep 32:738–751. https://doi.org/10.1039/c4np00167b

Jiang X, Ellabaan MMH, Charusanti P et al (2017) Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. Nat Commun 8:15784. https://doi.org/10.1038/ncomms15784

Joynt R, Seipke RF (2018) A phylogenetic and evolutionary analysis of antimycin biosynthesis. Microbiology 164:28–39. https://doi.org/10.1099/mic.0.000572

Kim B-J, Kim B-R, Kook Y-H, Kim B-J (2017a) Role of the DNA mismatch repair gene MutS4 in driving the evolution of Mycobacterium yongonense type I via homologous recombination. Front Microbiol 8:2578. https://doi.org/10.3389/fmicb.2017.02578

Kim B-J, Kim G-N, Kim B-R et al (2017b) Phylogenetic analysis of Mycobacterium massiliense strains having recombinant rpoB gene laterally transferred from Mycobacterium abscessus. PLoS One 12:e0179237. https://doi.org/10.1371/journal.pone.0179237

Klümper U, Riber L, Dechesne A et al (2015) Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. ISME J 9:934–945. https://doi.org/10.1038/ismej.2014.191

Kutzner HJ, Waksman SA (1959) Streptomyces coelicolor Muller and Streptomyces violaceoruber Waksman and Curtis, two distinctly different organisms. J Bacteriol 78:528

Lee RS, Radomski N, Proulx J-F et al (2015) Population genomics of Mycobacterium tuberculosis in the Inuit. Proc Natl Acad Sci U S A 112:13609–13614. https://doi.org/10.1073/pnas.1507071112

Letek M, Ocampo-Sosa AA, Sanders M et al (2008) Evolution of the Rhodococcus equi vap pathogenicity island seen through comparison of host-associated vapA and vapB virulence plasmids. J Bacteriol 190:5797–5805. https://doi.org/10.1128/JB.00468-08

Letek M, González P, Macarthur I et al (2010) The genome of a pathogenic rhodococcus: cooptive virulence underpinned by key gene acquisitions. PLoS Genet 6:e1001145. https://doi.org/10.1371/journal.pgen.1001145

Letzel A-C, Li J, Amos GCA et al (2017) Genomic insights into specialized metabolism in the marine actinomycete Salinispora. Environ Microbiol 19:3660–3673. https://doi.org/10.1111/1462-2920.13867

Levillain F, Poquet Y, Mallet L et al (2017) Horizontal acquisition of a hypoxia-responsive molybdenum cofactor biosynthesis pathway contributed to Mycobacterium tuberculosis pathoadaptation. PLoS Pathog 13:e1006752. https://doi.org/10.1371/journal.ppat.1006752

MacArthur I, Anastasi E, Alvarez S et al (2017) Comparative genomics of Rhodococcus equi virulence plasmids indicates host-driven evolution of the vap Pathogenicity Island. Genome Biol Evol 9:1241–1247. https://doi.org/10.1093/gbe/evx057

Mandlik A, Swierczynski A, Das A, Ton-That H (2007) Corynebacterium diphtheriae employs specific minor pilins to target human pharyngeal epithelial cells. Mol Microbiol 64:111–124. https://doi.org/10.1111/j.1365-2958.2007.05630.x

Manivasagan P, Venkatesan J, Sivakumar K, Kim S-K (2014) Pharmaceutically active secondary metabolites of marine Actinobacteria. Microbiol Res 169:262–278. https://doi.org/10.1016/j.micres.2013.07.014

McDonald BR, Currie CR (2017) Lateral gene transfer dynamics in the ancient bacterial genus streptomyces. MBio 8:e00644-17. https://doi.org/10.1128/mBio.00644-17

McInerney JO, McNally A, O'Connell MJ (2017) Why prokaryotes have pangenomes. Nat Microbiol 2:17040. https://doi.org/10.1038/nmicrobiol.2017.40

McKean SC, Davies JK, Moore RJ (2007) Expression of phospholipase D, the major virulence factor of Corynebacterium pseudotuberculosis, is regulated by multiple environmental factors and plays a role in macrophage death. Microbiology 153:2203–2211. https://doi.org/10.1099/mic.0.2007/005926-0

Medini D, Donati C, Tettelin H et al (2005) The microbial pan-genome. Curr Opin Genet Dev 15:589–594. https://doi.org/10.1016/j.gde.2005.09.006

Miyoshi T, Miyairi N, Aoki H et al (1972) Bicyclomycin, a new antibiotic. I. Taxonomy, isolation and characterization. J Antibiot 25:569–575

Mortimer TD, Pepperell CS (2014) Genomic signatures of distributive conjugal transfer among mycobacteria. Genome Biol Evol 6:2489–2500. https://doi.org/10.1093/gbe/evu175

Neuenschwander SM, Ghai R, Pernthaler J, Salcher MM (2018) Microdiversification in genome-streamlined ubiquitous freshwater Actinobacteria. ISME J 12:185–198. https://doi.org/10.1038/ismej.2017.156

Nouioui I, Carro L, García-López M et al (2018) Genome-based taxonomic classification of the phylum Actinobacteria. Front Microbiol 9:2007. https://doi.org/10.3389/fmicb.2018.02007

Nudel K, Zhao X, Basu S et al (2018) Genomics of Corynebacterium striatum, an emerging multidrug-resistant pathogen of immunocompromised patients. Clin Microbiol Infect 24:1016.e7–1016.e13. https://doi.org/10.1016/j.cmi.2017.12.024

Otchere ID, Coscollá M, Sánchez-Busó L et al (2018) Comparative genomics of Mycobacterium africanum lineage 5 and lineage 6 from Ghana suggests distinct ecological niches. Sci Rep 8:11269. https://doi.org/10.1038/s41598-018-29620-2

Panda A, Drancourt M, Tuller T, Pontarotti P (2018) Genome-wide analysis of horizontally acquired genes in the genus Mycobacterium. Sci Rep 8:14817. https://doi.org/10.1038/s41598-018-33261-w

Papke RT, Zhaxybayeva O, Feil EJ et al (2007) Searching for species in haloarchaea. Proc Natl Acad Sci U S A 104:14092–14097. https://doi.org/10.1073/pnas.0706358104

Penn K, Jenkins C, Nett M et al (2009) Genomic islands link secondary metabolism to functional adaptation in marine Actinobacteria. ISME J 3:1193–1203. https://doi.org/10.1038/ismej.2009.58

Pertry I, Václavíková K, Depuydt S et al (2009) Identification of Rhodococcus fascians cytokinins and their modus operandi to reshape the plant. Proc Natl Acad Sci U S A 106:929–934. https://doi.org/10.1073/pnas.0811683106

Podell S, Gaasterland T, Allen EE (2008) A database of phylogenetically atypical genes in archaeal and bacterial genomes, identified using the DarkHorse algorithm. BMC Bioinformatics 9:419. https://doi.org/10.1186/1471-2105-9-419

Putnam ML, Miller ML (2007) Rhodococcus fascians in herbaceous perennials. Plant Dis 91:1064–1076. https://doi.org/10.1094/PDIS-91-9-1064

Reva O, Korotetskiy I, Ilin A (2015) Role of the horizontal gene exchange in evolution of pathogenic mycobacteria. BMC Evol Biol 15(Suppl 1):S2. https://doi.org/10.1186/1471-2148-15-S1-S2

Ruiz JC, D'Afonseca V, Silva A et al (2011) Evidence for reductive genome evolution and lateral acquisition of virulence functions in two Corynebacterium pseudotuberculosis strains. PLoS One 6:e18551. https://doi.org/10.1371/journal.pone.0018551

Sakaguchi K (1990) Invertrons, a class of structurally and functionally related genetic elements that includes linear DNA plasmids, transposable elements, and genomes of adeno-type viruses. Microbiol Rev 54:66–74

Sánchez-Romero MA, Casadesús J (2014) Contribution of phenotypic heterogeneity to adaptive antibiotic resistance. Proc Natl Acad Sci U S A 111:355–360. https://doi.org/10.1073/pnas.1316084111

Sanchini A, Semmler T, Mao L et al (2016) A hypervariable genomic island identified in clinical and environmental Mycobacterium avium subsp. hominissuis isolates from Germany. Int J Med Microbiol 306:495–503. https://doi.org/10.1016/j.ijmm.2016.07.001

Sangal V, Hoskisson PA (2016) Evolution, epidemiology and diversity of Corynebacterium diphtheriae: new perspectives on an old foe. Infect Genet Evol 43:364–370. https://doi.org/10.1016/j.meegid.2016.06.024

Sapriel G, Konjek J, Orgeur M et al (2016) Genome-wide mosaicism within Mycobacterium abscessus: evolutionary and epidemiological implications. BMC Genomics 17:118. https://doi.org/10.1186/s12864-016-2448-1

Schatz A, Bugle E, Waksman SA (1944) Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative Bacteria.∗†. Proc Soc Exp Biol Med 55:66–69. https://doi.org/10.3181/00379727-55-14461

Sechler AJ, Tancos MA, Schneider DJ et al (2017) Whole genome sequence of two Rathayibacter toxicus strains reveals a tunicamycin biosynthetic cluster similar to Streptomyces chartreusis. PLoS One 12:e0183005. https://doi.org/10.1371/journal.pone.0183005

Seipke RF (2015) Strain-level diversity of secondary metabolism in Streptomyces albus. PLoS One 10:e0116457. https://doi.org/10.1371/journal.pone.0116457

Sengupta S, Chattopadhyay MK, Grossart H-P (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 4:47. https://doi.org/10.3389/fmicb.2013.00047

Sharaf A, Mercati F, Elmaghraby I et al (2017) Functional and comparative genome analysis of novel virulent actinophages belonging to Streptomyces flavovirens. BMC Microbiol 17:51. https://doi.org/10.1186/s12866-017-0940-7

Sheppard SK, Guttman DS, Fitzgerald JR (2018) Population genomics of bacterial host adaptation. Nat Rev Genet 19:549–565. https://doi.org/10.1038/s41576-018-0032-z

Silby MW, Winstanley C, Godfrey SAC et al (2011) Pseudomonas genomes: diverse and adaptable. FEMS Microbiol Rev 35:652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Skordalakes E, Brogan AP, Park BS et al (2005) Structural mechanism of inhibition of the rho transcription termination factor by the antibiotic bicyclomycin. Structure 13:99–109. https://doi.org/10.1016/j.str.2004.10.013

Snel B, Bork P, Huynen MA (2002) Genomes in flux: the evolution of archaeal and proteobacterial gene content. Genome Res 12:17–25. https://doi.org/10.1101/gr.176501

Stackebrandt E, Schumann P (2006) Introduction to the taxonomy of Actinobacteria. In: Dworkin M, Falkow S, Rosenberg E et al (eds) The prokaryotes, Archaea. Bacteria: Firmicutes, Actinomycetes, vol Volume 3. Springer, New York, NY, pp 297–321

Stucki D, Brites D, Jeljeli L et al (2016) Mycobacterium tuberculosis lineage 4 comprises globally distributed and geographically restricted sublineages. Nat Genet 48:1535–1543. https://doi.org/10.1038/ng.3704

Takhaveev V, Heinemann M (2018) Metabolic heterogeneity in clonal microbial populations. Curr Opin Microbiol 45:30–38. https://doi.org/10.1016/j.mib.2018.02.004

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In: American Mathematical Society: lectures on mathematics in the life sciences. American Mathematical Society, Providence, RI, pp 57–86

Thoma L, Muth G (2015) The conjugative DNA-transfer apparatus of Streptomyces. Int J Med Microbiol 305:224–229. https://doi.org/10.1016/j.ijmm.2014.12.020

Thoma L, Dobrowinski H, Finger C et al (2015) A multiprotein DNA translocation complex directs intramycelial plasmid spreading during Streptomyces conjugation. MBio 6:e02559–e02514. https://doi.org/10.1128/mBio.02559-14

Tolba S, Egan S, Kallifidas D, Wellington EMH (2002) Distribution of streptomycin resistance and biosynthesis genes in streptomycetes recovered from different soil sites. FEMS Microbiol Ecol 42:269–276. https://doi.org/10.1111/j.1574-6941.2002.tb01017.x

Topino S, Galati V, Grilli E, Petrosillo N (2010) Rhodococcus equi infection in HIV-infected individuals: case reports and review of the literature. AIDS Patient Care STDs 24:211–222. https://doi.org/10.1089/apc.2009.0248

Trejo WH (1970) An evaluation of some concepts and criteria used in the speciation of streptomycetes. Trans N Y Acad Sci 32:989–997. https://doi.org/10.1111/j.2164-0947.1970.tb02991.x

Trost E, Blom J, Soares S de C et al (2012) Pangenomic study of Corynebacterium diphtheriae that provides insights into the genomic diversity of pathogenic isolates from cases of classical diphtheria, endocarditis, and pneumonia. J Bacteriol 194:3199–3215. https://doi.org/10.1128/JB.00183-12

True JR, Carroll SB (2002) Gene co-option in physiological and morphological evolution. Annu Rev Cell Dev Biol 18:53–80. https://doi.org/10.1146/annurev.cellbio.18.020402.140619

Uchiya K-I, Tomida S, Nakagawa T et al (2017) Comparative genome analyses of Mycobacterium avium reveal genomic features of its subspecies and strains that cause progression of pulmonary disease. Sci Rep 7:39750. https://doi.org/10.1038/srep39750

Udwary DW, Zeigler L, Asolkar RN et al (2007) Genome sequencing reveals complex secondary metabolome in the marine actinomycete Salinispora tropica. Proc Natl Acad Sci U S A 104:10376–10381. https://doi.org/10.1073/pnas.0700962104

Valero-Rello A, Hapeshi A, Anastasi E et al (2015) An invertron-like linear plasmid mediates intracellular survival and virulence in bovine isolates of Rhodococcus equi. Infect Immun 83:2725–2737. https://doi.org/10.1128/IAI.00376-15

Van Goethem MW, Pierneef R, Bezuidt OKI et al (2018) A reservoir of "historical" antibiotic resistance genes in remote pristine Antarctic soils. Microbiome 6:40. https://doi.org/10.1186/s40168-018-0424-5

Ventura M, Canchaya C, Tauch A et al (2007) Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev 71:495–548. https://doi.org/10.1128/MMBR.00005-07

Viana MVC, Figueiredo H, Ramos R et al (2017) Comparative genomic analysis between Corynebacterium pseudotuberculosis strains isolated from buffalo. PLoS One 12:e0176347. https://doi.org/10.1371/journal.pone.0176347

Vinatzer BA, Monteil CL, Clarke CR (2014) Harnessing population genomics to understand how bacterial pathogens emerge, adapt to crop hosts, and disseminate. Annu Rev Phytopathol 52:19–43. https://doi.org/10.1146/annurev-phyto-102313-045907

Vior NM, Lacret R, Chandra G et al (2018) Discovery and biosynthesis of the antibiotic Bicyclomycin in distantly related bacterial classes. Appl Environ Microbiol 84:AEM.02828-17. https://doi.org/10.1128/AEM.02828-17

von Bargen K, Haas A (2009) Molecular and infection biology of the horse pathogen Rhodococcus equi. FEMS Microbiol Rev 33:870–891. https://doi.org/10.1111/j.1574-6976.2009.00181.x

Whitaker RJ, Banfield JF (2006) Population genomics in natural microbial communities. Trends Ecol Evol 21:508–516. https://doi.org/10.1016/j.tree.2006.07.001

Williams M, Mizrahi V, Kana BD (2014) Molybdenum cofactor: a key component of Mycobacterium tuberculosis pathogenesis? Crit Rev Microbiol 40:18–29. https://doi.org/10.3109/1040841X.2012.749211

Wilson DJ (2012) Insights from genomics into bacterial pathogen populations. PLoS Pathog 8:e1002874. https://doi.org/10.1371/journal.ppat.1002874

Woodruff HB (2014) Selman A. Waksman, winner of the 1952 Nobel Prize for physiology or medicine. Appl Environ Microbiol 80:2–8. https://doi.org/10.1128/AEM.01143-13

Youngblut ND, Wirth JS, Henriksen JR et al (2015) Genomic and phenotypic differentiation among Methanosarcina mazei populations from Columbia River sediment. ISME J 9:2191–2205. https://doi.org/10.1038/ismej.2015.31

Zhou Z, Gu J, Li Y-Q, Wang Y (2012) Genome plasticity and systems evolution in Streptomyces. BMC Bioinformatics 13(Suppl 10):S8. https://doi.org/10.1186/1471-2105-13-S10-S8

Ziemert N, Lechner A, Wietz M et al (2014) Diversity and evolution of secondary metabolism in the marine actinomycete genus Salinispora. Proc Natl Acad Sci U S A 111:E1130–E1139. https://doi.org/10.1073/pnas.1324161111

Zumla A, George A, Sharma V et al (2015) The WHO 2014 global tuberculosis report—further to go. Lancet Glob Health 3:e10–e12. https://doi.org/10.1016/S2214-109X(14)70361-4